Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3670–3675
Bioorganic & Medicinal Chemistry Letters

A new class of histamine H_3 receptor antagonists derived from ligand based design

Olivier Roche and Rosa María Rodríguez Sarmiento*

F. Hoffmann-La Roche Ltd, Pharmaceutical Research Basel, Discovery Chemistry, CH-4070 Basel, Switzerland

Received 12 March 2007; revised 13 April 2007; accepted 15 April 2007
Available online 25 April 2007

Abstract—Design and synthesis of highly potent and selective non-imidazole inverse agonists for the histamine H_3 receptor is described. The study validates a new pharmacophore model based on the merging of two previously described models. It also demonstrates that the removal of the basic center potentially interacting with ASP3.32 and common to both models leads to loss of activity, whereas the replacement of the second basic center by an acceptor retains the potency.

Histamine is a central modulator of physiological processes acting both in the central nervous system (CNS) and the periphery through four distinct receptors (H_1–H_4) known to date.[1,2] Histamine H_1 and H_2 receptor antagonists are already used in the clinic to treat allergic asthma and excess gastric acid production, respectively. The newly discovered H_4 receptor seems to have a role in regulating inflammatory responses.[3] The H_3 receptor, mainly located in the CNS, is a presynaptic autoreceptor that does not only modulate the production and the release of histamine from histaminergic neurons[4] but also regulates the release of other neurotransmitters in both the central and peripheral nervous systems.[5–7] Another feature of the H_3 receptor is its high constitutive activity.[8,9] H_3 antagonists/inverse agonists have been primarily studied in cognitive models for Alzheimer's disease, attention-deficit disorder, and schizophrenia.[10,11] Pharmacological data also suggest a potential role for H_3 antagonists and/or inverse agonists in the control of feeding, appetite, and body weight, and support the role of H_3 receptor in obesity.[12,13]

An analysis of known low nanomolar H_3 antagonists published in patents and literature has been made in order to derive a three-dimensional pharmacophore model. Since imidazole-containing compounds could have potential liability toward cytochrome-P450 (CYP) drug metabolizing enzymes, the imidazole-based H_3 antagonists were discarded.[14] As it has been shown that basic amine-based molecules have different binding modes than imidazole-based ones, a large part of the modeling information cannot be used.[15–19] The focus has then been on the basic amine-containing compounds that are postulated to interact with the highly conserved ASP3.32.[16] During the manual alignment of the molecules, we can observe two distinct pharmacophore distributions (Fig. 1). Each model consists of four pharmacophoric features. Three of the features are shared by both models, namely a distal positive charge, an electron rich position, and a central aromatic ring. The fourth feature can be either a second basic amine exemplified with a potent Johnson & Johnson compound (model 1)[20] or another aromatic contained in many Abbott molecules coming from A. Hancock group like A-349821 (model 2),[21] but also later ABT-239.[22]

Both types of molecules have been extensively described separately and, at the time of the study, no molecules have been disclosed combining all the features of the combined pharmacophore. Nevertheless, since then, research teams at Johnson & Johnson have published various chemotypes supporting our pharmacophore hypothesis.[23–25] Our study describes the rational approach followed at Roche to generate new chemotypes validating our assumption. Considering the large overlapping of the pharmacophores, we assume a common binding mode, which will be driven by the interaction between the common basic amine and the conserved aspartate in helix 3 (ASP3.32). Thus, we

Keywords: Histamine H_3 receptor; SAR; Pharmacophore model; De novo design; Skelgen.
* Corresponding author. Tel.: +41 61 6883585; fax: +41 61 6886459; e-mail: rosa_maria.rodriguez_sarmiento@roche.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.056

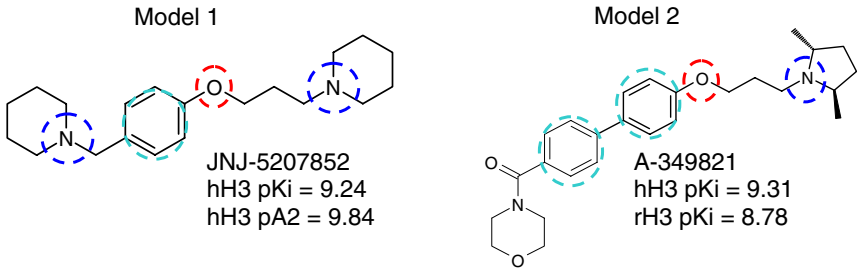


Figure 1. Two pharmacophore models derived from known antagonists of the histamine H_3 receptors. The pharmacophoric features are displayed as dashed circles: blue, positive charge (basic amine); red, electron rich; cyan, aromatic/lipophilic.

designed a new pharmacophore model consisting of five features: two aromatics, two positively charged moieties, and one electron rich feature. We included the electron rich feature because our entire set of reference molecules contains an ether linker at the same position, but the available SAR does not allow us to be sure it is fully mandatory. To provide starting points for the chemistry effort, we use the de novo program Skelgen.[26–28] The program automatically builds new molecules from fragment libraries that match the three-dimensional pharmacophoric constraints. It provides hundred of possible molecules that have been manually clustered. This leads to four templates, which share the same pharmacophores but have different connectivity patterns (Fig. 2). The chemical synthesis has been developed around the connective pattern of template two in order to validate the new pharmacophore.

Synthesis of 4-amino quinazoline of type **1** was better accomplished as indicated in Scheme 1, starting with the O-alkylation of commercially available nitro phenol (**3**) with benzyl chloride. The reductive amination of the

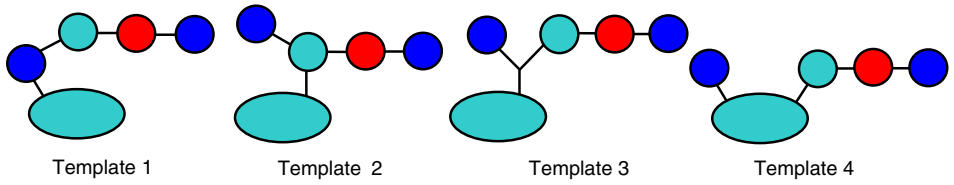
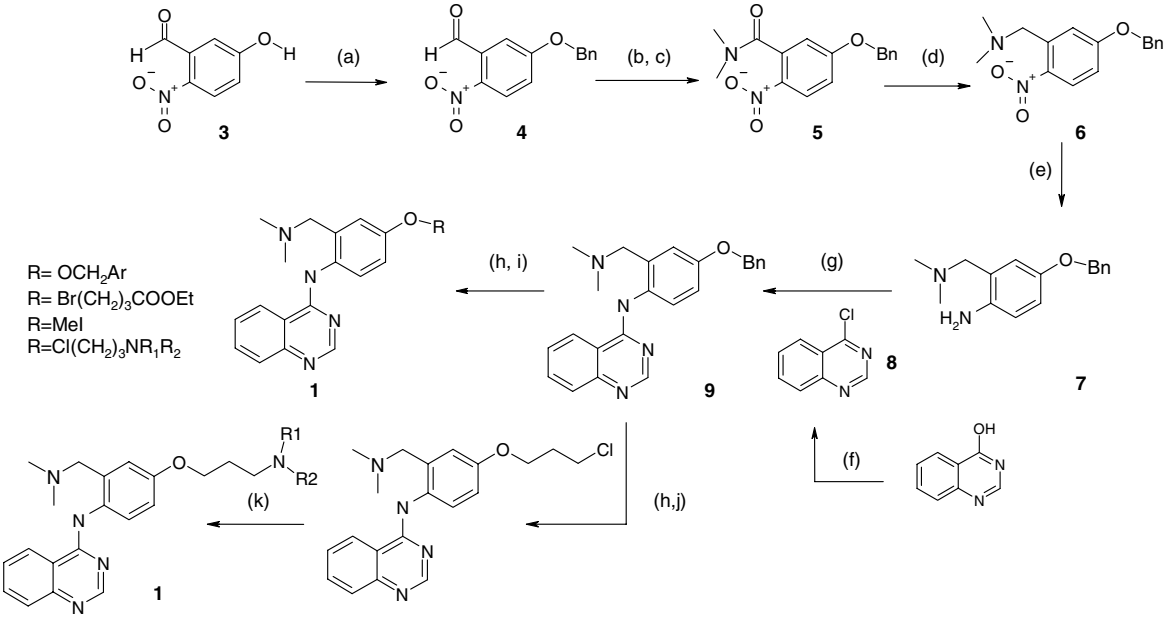
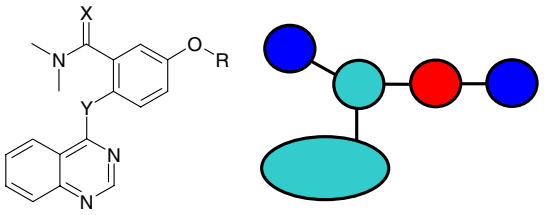


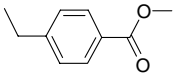
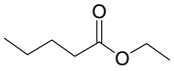
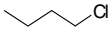
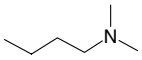
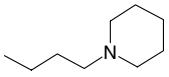
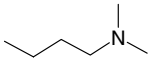
Figure 2. Projection scheme of the various connectivity patterns between the pharmacophore features suggested by Skelgen. Blue, positive charge (basic amine); red, electron rich; cyan, aromatic/lipophilic.



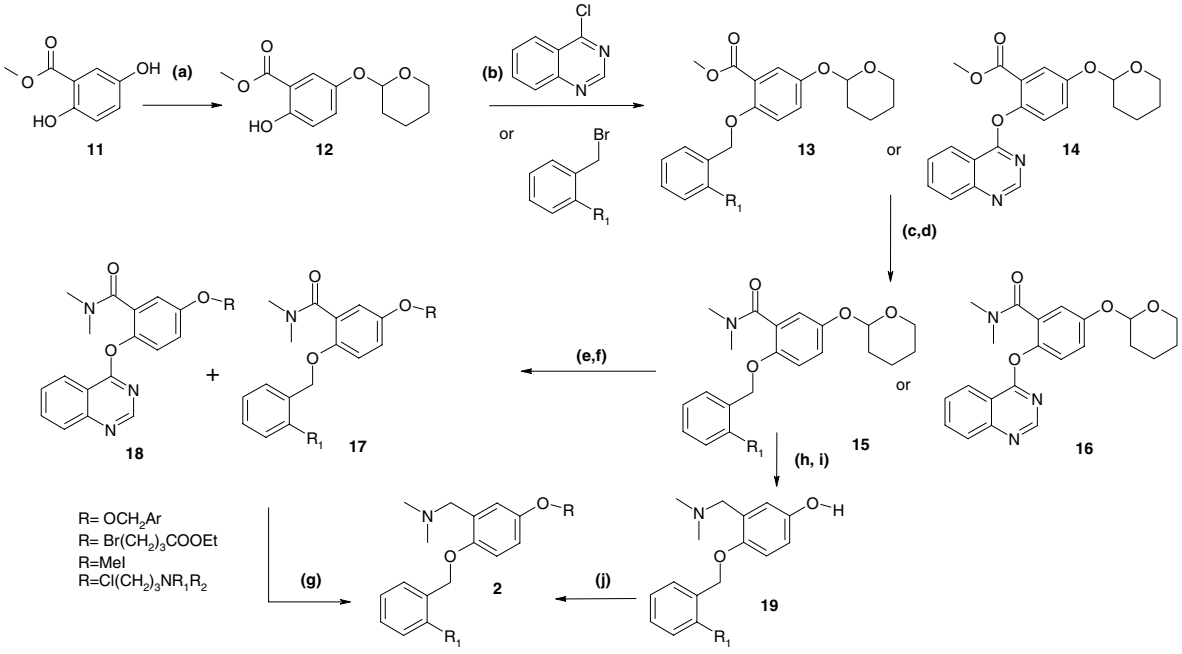
Scheme 1. Reagents and conditions: (a) BnCl (excess), K_2CO_3, DMF, 110 °C, 4 h, 100%; (b) $KMnO_4$, acetone/water (1:1), 5 h, 98%; (c) HBTU, dimethylamine hydrochloride, TEA, DMF, 3 h, 89%; (d) BH_3·THF, THF, 70 °C, 8 h, 90% (complex); (e) Pt–C 10%, H_2, EtOAc, 18 h, 80%; (f) $POCl_3$, 100 °C, microwave, 15 min; (g) **8**, EtOH, DIPEA, reflux, 12 h, 85%; (h) Pd (10% C), EtOH, H_2, rt, overnight, 83%; (i) RX, K_2CO_3, DMF, 18 h, 60 °C, 40–10% or two-step procedure with (j) $Br(CH_2)_3Cl$, Cs_2CO_3, acetone, rt, 5 h, 93%; (k) Corresponding amine, EtOH, reflux, 5 h, 65–90%.

Table 1. Binding data on human histamine receptors H_1, H_2, H_3 for the compounds of series 1



Compound	X	R	Y	hH_3 K_i (nM)	hH_3% inhibition[a]	hH_1% inhibition[a]	hH_2% inhibition[a]
ABT-239[22]				2.4			
JNJ-5207852[20]				1.4			
1a	H	H	N		11.6%		
1b	H		N		31%		
1c	H		N		28%		
1d	H		N		49%		
1e	H		N	18		−15%	−2%
1f	H		N	0.3	—	−2.5%	2.4%
18a	O		O	1021			

[a] Percentage of inhibition measured at 10 μM.

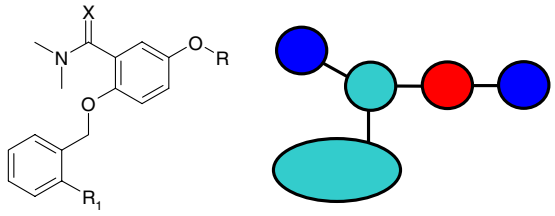


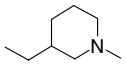
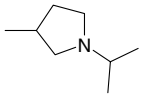
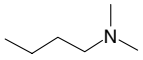
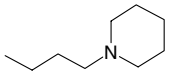
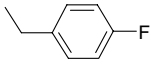
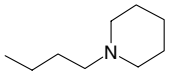
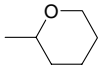
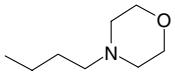
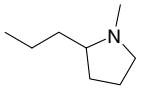
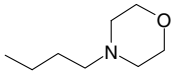
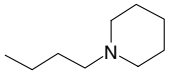
Scheme 2. Reagents and conditions: (a) DHP, PPTS, CH_2Cl_2, 12 h, 97%; (b) 4-chloro-quinazoline **8**, Cs_2CO_3, DMF, 90 °C, 4 h, 90% or $BrCH_2Ar$, Cs_2CO_3, 40 °C, acetone, 4 h, 100%; (c) LiOH, THF, H_2O, 40 °C, 18 h, 100%; (d) CDI, $HN(CH_3)_2$·HCl, DMF, 60 °C, or HBTU, Et_3N, $HN(CH_3)_2$·HCl, DMF, 2 h, 90–70%; (e) pTsOH, MeOH, 60 °C, 8 h, 84%; (f) K_2CO_3, RX, DMF, 100 °C, 2 h, 90–70%; (g) BH_3·THF THF, 90 °C, 4 h, 63%; (h) $LiAlH_4$, THF, 40 °C, 18 h, 70%; (i) pTsOH, MeOH, 60 °C, 8 h, 69%; (j) RX, K_2CO_3, DMF, 100 °C, 3 h, 20–70%.

α-nitroaldehyde (**4**) gave low yields and an alternative route via the reduction of the corresponding amide was followed. The aldehyde (**4**) was oxidized to the acid to obtain via HBTU coupling the *N*,*N*-dimethyl amide (**5**). Reduction of the amide was performed with borane–THF complex to obtain the amine (**6**) as a borane complex. After reduction of the nitro group in the presence of the benzyl group with platinum as catalyst, the compound **7** was obtained free of borane. Alkylation of the nitrogen with 4-chloro-quinazoline (**8**) (obtained from the treatment of commercially available quinazolin-4-ol with $POCl_3$) was performed in ethanol. The use of DIPEA allows the reaction to proceed rapidly and more cleanly toward **9**. Removal of the benzyl group gives the phenol that can react with a range of alkylating agents in the presence of K_2CO_3 to obtain compounds of type **1** described in Table 1. In cases where direct alkylation did not provide high yields, a two-step procedure can be used instead with a first alkylation with 1-bromo-3-chloropropane using Cs_2CO_3 to obtain the *O*-alkylchloride that can be heated with the corresponding amine to prepare **1**. Alternative routes with the use of 4-fluorophenol or 2,5-dihydroxybenzaldehyde gave undesired byproducts and lower yields.

In the case of compounds of type **2** (4-oxo quinazolines or benzyloxy compounds), the most successful synthetic route starts with the regioselective alkylation of the commercially available 2,5-dihydroxy-benzoic acid (**11**) with THP under acidic conditions to obtain **12**.[29] Alkylation with 4-chloro-quinazoline (**8**) or with the corresponding alkylbromide with Cs_2CO_3 gives **13** or **14**, respectively. Hydrolysis of the ester using LiOH and amide formation with $HNMe_2{\cdot}HCl$ using CDI gives

Table 2. Binding data on human histamine receptors H_1, H_2, H_3 for the compounds of series 2



Compound	X	R	R_1	hH_3 K_i (nM)	hH_3% inhibition[a]	hH_1% inhibition[a]	hH_2% inhibition[a]
2a	H		Cl	150			
2b	H		Cl	88			
2c	H		Cl	135			
2d	H		Cl	2.4		—	—
17a	O		Cl		33%		
17b	O		Cl	47		8%	
17c	O		Cl		17%		
17d	O		Cl	1100			
17e	O		Cl	1810			
17f	O		OMe	1393	73.4%		
17g	O		OMe	12			

[a] Percentage of inhibition measured at 10 μM.

the amides **15** or **16**. In both cases removal of the THP and O-alkylation with an appropriate alkylating agent gave compounds **17** or **18**. Reduction of the amide group on **17** or **18** to the corresponding amines using borane–tetrahydrofuran complex gives reasonable yields except on compounds for type **17** when a benzyl or methyl group is present (R = OCH_2Ar and R = Me). An alternative way to obtain the desired amine **2** was through the reduction of compound **15**, using $LiAlH_4$. Removal of the THP group under acidic conditions gives the phenol **19** that can be alkylated with K_2CO_3 and the corresponding halogenated group. The compounds synthesized by this route (Scheme 2) are exemplified in Table 2.

The H_3 activities presented in Tables 1 and 2 are binding data measured by displacement of [^{3}H](R)-α-methylhistamine (RAMH).[30] For selected compounds, human H_1 and H_2 subtype affinity has been measured using [^{3}H]pyrilamine and [^{3}H]tiotidine, respectively (see Tables 1 and 2).[31] The SAR explored by the molecules shown in these tables validate the new pharmacophore model since compounds **1e**, **1f**, and **2a**–**2d** carrying 2 basic centers and a lipophilic substituent altogether are highly active at the H_3 receptor. When we remove the distal basic amine in compounds **1a**–**1d**, potentially interacting with ASP3.32, the activity at the H_3 receptor is completely lost. For the molecules **18a**, **17b**, **17e**, and **17g** where the proximal basic center has been eliminated by generating the amide, part of activity can be conserved. Especially, compounds **17b** and **17g** have a K_i of 42 and 12 nM at the H_3 subtype, respectively. From our results, it is likely that both the proximal basic nitrogen and the carbonyl oxygen of the amide bond interact with the same residue. A potential candidate residue for this type of interaction could be Glu5.46, which can be both a hydrogen bond donor (carbonyl, **17g**) and acceptor (basic amine, **2d**).

From the potent compounds **1e**, **1f**, **17b** tested against human H_1 and H_2 receptors we confirmed that our series are selective for the H_3 subtype. The most promising compound **1f** has been further profiled in terms of functionality and species selectivity. It has been tested in a GTPγS functional assay and was found to be a potent inverse agonist with an EC_{50} of 0.2 nM. Moreover, **1f** also displays a high affinity toward the rat H_3 receptor with a K_i of 9.8 nM qualifying the molecule for further in vivo experiments. Preliminary safety data show that **1f** does not block the hERG channel.[32]

In conclusion, applying a new pharmacophore model, we have discovered highly potent and selective inverse agonists for both human and rat H_3 receptors. In this study, we have exemplified only one of the connection patterns proposed by Skelgen. Based on these promising results, other chemotypes will be explored.

Acknowledgments

It is with real pleasure that we wish to thank all our collaborators whose contributions made the described work possible and so enjoyable, especially Dr. Silvia. Gatti McArthur, Sandra Grall-Ulsemer, Regine Denu, Dr. Harald Mauser, Dr. Wolfgang Guba, and Dr. M. Nettekoven.

References and notes

1. Sneader, W. *Drug News Perspect.* **2001**, *14*, 618.
2. Hough, L. B. *Mol. Pharmacol.* **2001**, *59*, 415.
3. Thurmond, R. L.; Desai, P. J.; Dunford, P. J.; Fung-Leung, W.-P.; Hofstra, C. L.; Jiang, W.; Nguyen, S.; Riley, J. P.; Sun, S.; Williams, K. N.; Edwards, J. P.; Karlsson, L. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 404.
4. Arrang, J. M.; Garbarg, M.; Schwartz, J. C. *Neuroscience* **1987**, *23*, 149.
5. Schlicker, E.; Kathmann, M. In *The Histamine H3 Receptor; a Target for New Drugs*; Leurs, R., Timmerman, H., Eds., 1st ed.; Elservier Science B.V.: Amsterdam, 1998; pp 13–26.
6. Stark, H. *Expert Opin. Ther. Pat.* **2003**, *13*, 851.
7. Leurs, R.; Blandina, P.; Tedford, C.; Timmerman, H. *Trends Pharmacol. Sci.* **1998**, *19*, 177.
8. Rouleau, A.; Ligneau, X.; Tardivel-Lacombe, J.; Morisset, S.; Gbahou, F.; Schwartz, J. C.; Arrang, J. M. Brit. *J. Pharmacol.* **2002**, *135*, 383.
9. Takahashi, K.; Tokita, S.; Kotani, . *J. Pharmacol. Exp. Ther.* **2003**, *307*, 213.
10. Witkin, J. M.; Nelson, D. L. *Pharmacol. Therapeut.* **2004**, *103*, 1.
11. Alguacil, L. F.; Perez-Garcia, C. *Curr. Drug Targets CNS Neurol. Disord.* **2003**, *2*, 303.
12. Hancock, A. A.; Brune, M. E. *Expert Opin. Invest. Drugs* **2005**, *14*, 223.
13. Hancock, A. A. *Curr. Opin. Invest. Drugs* **2003**, *4*, 1190.
14. Yang, R.; Hey, J. A.; Aslanian, R.; Rizzo, C. A. *Pharmacology* **2002**, *66*, 128.
15. Rivara, S.; Mor, M.; Bordi, F.; Silva, C.; Zuliani, V.; Vacondio, F.; Morini, G.; Plazzi, P. V.; Carrupt, P.-A.; Testa, B. *Drug Des. Discov.* **2003**, *18*, 65.
16. Yao, B. B.; Hutchins, C. W.; Carr, T. L.; Cassar, S.; Masters, J. N.; Bennani, Y. L.; Esbenshade, T. A.; Hancock, A. A. *Neuropharmacology* **2003**, *44*, 773.
17. Stark, H.; Sippl, W.; Ligneau, X.; Arrang, J. M.; Ganellin, C. R.; Schwartz, J. C.; Schunack, W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 951.
18. De Esch, I. J.; Mills, J. E.; Perkins, T. D.; Romeo, G.; Hoffmann, M.; Wieland, K.; Leurs, R.; Menge, W. M.; Nederkoorn, P. H.; Dean, P. M.; Timmerman, H. *J. Med. Chem.* **2001**, *44*, 1666.
19. De Esch, I. J. P.; Timmerman, H.; Menge, W. M. P. B.; Nederkoorn, P. H. J. *Arch. Pharm. (Weinheim, Germany)* **2000**, *333*, 254.
20. Apodaca, R.; Dvorak, C. A.; Xiao, W.; Barbier, A. J.; Boggs, J. D.; Wilson, S. J.; Lovenberg, T. W.; Carruthers, N. I. *J. Med. Chem.* **2003**, *46*, 3938.
21. Faghih, R.; Dwight, W.; Bao Pan, J.; Fox, G. B.; Krueger, K. M.; Esbenshade, T. A.; McVey, J. M.; Marsh, K.; Bennani, Y. L.; Hancock, A. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1325.
22. Cowart, M.; Pratt, J. K.; Stewart, A. O.; Bennani, Y. L.; Esbenshade, T. A.; Hancock, A. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 689.
23. Keith, J. M.; Gomez, L. A.; Letavic, M. A.; Ly, K. S.; Jablonowski, J. A.; Seierstad, M.; Barbier, A. J.; Wilson, S. J.; Boggs, J. D.; Fraser, I. C.; Mazur, C.; Lovenberg, T. W.; Carruthers, N. I. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 702.

24. Letavic, M. A.; Keith, J. M.; Jablonowski, J. A.; Stocking, E. M.; Gomez, L. A.; Ly, K. S.; Miller, J. M.; Barbier, A. J.; Bonaventure, P.; Boggs, J. D.; Wilson, S. J.; Miller, K. L.; Lord, B.; McAllister, H. M.; Tognarelli, D. J.; Wu, J.; Abad, M. C.; Schubert, C.; Lovenberg, T. W.; Carruthers, N. I. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1047.
25. Letavic, M. A.; Keith, J. M.; Ly, K.; Barbier, A. J.; Boggs, J. D.; Wilson, S. J.; Lord, B.; Lovenberg, T. W.; Carruthers, N. I. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2566.
26. Dean, P. M.; Firth-Clark, S.; Harris, W.; Kirton, S. B.; Todorov, N. P. *Expert Opin. Drug Disc.* **2006**, *1*, 179.
27. Lloyd, D. G.; Buenemann, C. L.; Todorov, N. P.; Manallack, D. T.; Dean, P. M. *J. Med. Chem.* **2004**, *47*, 493.
28. Stahl, M.; Todorov Nikolay, P.; James, T.; Mauser, H.; Boehm, H.-J.; Dean Philip, M. *J. Comput. Aided Mol. Des.* **2002**, *16*, 459.
29. Tori, M.; Hamaguchi, T.; Sagawa, K.; Sono, M.; Asakawa, Y. *J. Org. Chem.* **1996**, *61*, 5362.
30. Saturation binding experiments were performed using HR3-CHO membranes prepared as described in: (a) Takahashi, K.; Tokita, S.; Kotani, H. *J. Pharmacol. Exp. Ther.* **2003**, *307*, 213; All compounds were tested at a single concentration in duplicate. Compounds that showed an inhibition of [^{3}H]RAMH by more than 50% were tested again to determine IC_{50} in a serial dilution experiment. K_i's were calculated from IC_{50} based on Cheng–Prusoff equation: (b) Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099; (c) For a more detailed description see: Gatti, S.; Hertel, C.; Nettekoven, M.; Plancher, J.-M.; Raab, S.; Roche, O.; Rodriguez-Sarmiento, R.-M. *PCT Int. Appl.* **2005**, WO2005117865.
31. Membranes and protocols originated from Euroscreen. For more information, see: characterization of histaminergic receptors *Current Protocols in Pharmacology*, Ed. J. Wiley & Sons, **2006**; Vol. 1, Chapter 19, pp 1–19.
32. Compound **1f** showed 30% inhibition at 10 μM concentration at the hERG channel (IC_{50} > 10 μM).